The disruption of *JEN1* from *Candida albicans* impairs the transport of lactate

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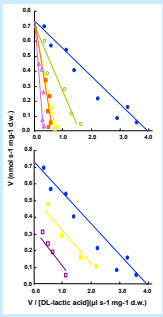
Introduction

Lactic acid-grown cells of *Candida albicans* display activity for a monocarboxylate permease. The present work aims at the functional characterization of the gene(s) coding for the lactate permease in this yeast. In yeasts, and in fungi in general, until the moment only two genes coding for short-chain carboxylate permeases have been identified: the monocarboxylate/proton symporter *JEN1* gene from *Saccharomyces cerevisiae* (Casal *et al*., 1999) and the dicarboxylate/proton symporter *MAE1* gene of *Schizosaccharomyces pombe* (Grobler *et al*., 1995). In a search made in the Stanford Genome Technology Center using the BLAST program an ORF homologous to *JEN1* was found. In the present work the functional analysis the gene *CaJEN1* of *Candida albicans* was performed.

Results

Lactic acid-grown cells of *C. albicans* displayed activity for a monocarboxylate permease



DL-lactic acid, pH 5.0

A)
- absence of other substrates
- 0,5 mM propionic acid
- 2 mM D- lactic acid
- 2 mM L-lactic acid
- 1 mM pyruvic acid

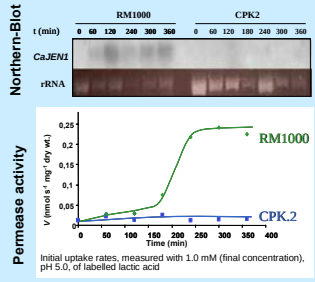
B)
- absence of other substrates
- 2 mM acetic acid
- 4 mM acetic acid

Kinetic parameters pH 5.0

V_{max} = 0,85 ± 0.06 nmol/s/mg d.w.
K_m = 0.33 ± 0.09 mM lactic acid

CaJen1 permease was shared by lactate, propionate, pyruvate

Induction of *CaJEN1* in lactic acid-induced cells



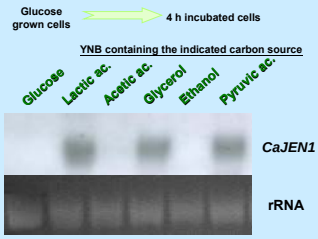
Relevant genotype of the strains:

C. albicans RM1000 - wild-type strain
C. albicans CPK.2 – *jen1::HIS/jen1::URA*

C. albicans strain	RM1000	CPK2
CaJEN1 mRNA	+	-
Jen1p activity	+	-

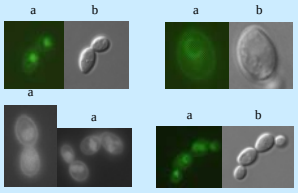
No *CaJEN1* mRNA or lactate permease activity were detected in CPK2 strain

CaJEN1 expression in different media



CaJEN1 was expressed after four hours induction in media containing lactic acid, glycerol or pyruvic acid

Localization of CaJen1p in lactic acid-induced cells of *C. albicans*



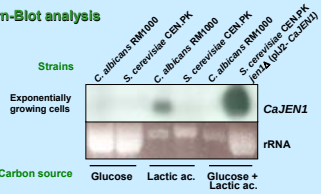
a - Epifluorescence microscopy b - Phase contrast microscopy

Cells induced for four hours in YNB lactic acid

CaJen1p was targeted to the plasma membrane in lactic acid induced cells of *C. albicans*

Expression of *CaJEN1* in *S. cerevisiae*

Northern-Blot analysis



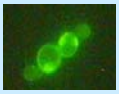
CaJEN1 is transcribed in *S. cerevisiae*

Lactate transport analysis : *jen1Δ* cells did not recorver the activity for the permease

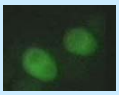
CaJEN1 was not functional in *S. cerevisiae*

Localization of *CaJEN1* in *S. cerevisiae*

5 hours induction in medium without methionine



S. cerevisiae pUG35-CaJEN1 fluorescence in the plasma membrane



S. cerevisiae pUG35 fluorescence in the cytoplasm

CaJen1p was targeted to the plasma membrane, under the control of the Met25 promoter

Alignment of JEN1p sequences

Organism	Aminoacid sequence	Accession
Beauveria bassiana	287ALEQCPSNARGLMSGILQQGYSFGY311	AY187631
Metarhizium anisopliae	182ALEQCPSNARGLMSGILQQGYSFGY206	AY125927
Neurospora crassa	183ALENSPVDARGLMSGILQQGYAFGY207	AL353819
Thermoplasma acidophilum	128AMESLPAKARGWVSGLIQGGVPTGY144	AL445064
Kluyveromyces lactis	211ALENAPNKAKSILSGIFQEGYAFGX235	AL428866
Kluyveromyces lactis	192AIEDAPVKARSFLSGLFFTAYAMGF216	AL426631
Saccharomyces cerevisiae	253AIEDAPVKARSFLSGLFFSAYAMGF277	U24155
Candida albicans	204ALEQQPIAARSVLSGLFLPGYCFGY228	19-2514*
Identity	A-E--P---A- - - -SG- - - - -Y- -G-	
Consensus	ALE3APVKAR2LLSG2FQQGYAFGY	

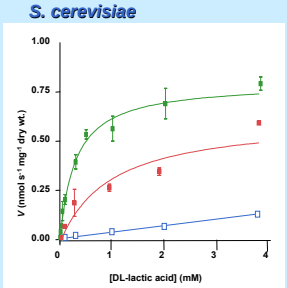
* contig number obtained from the last assembly of the *C. albicans* genome in the Stanford Genome Technology Center

S – is encoded by a CTG codon, which encodes for a Serine in *C. albicans* and a Leucine in *S. cerevisiae* (Santos *et al*., 1993)

Site directed mutagenesis

CTG - *C. albicans* → Serine
S. cerevisiae → Leucine
⇨
TTC - *C. albicans* → Serine
S. cerevisiae → Serine

Functionality of the CaJen1p (217L→S) mutant in *S. cerevisiae*



C. albicans RM1000

S. cerevisiae p416-CaJen1-m

Kinetic parameters pH 5.0

V_{max}= 0.63 nmol/s/mg d.w.
K_m = 0.98 mM lactic acid

C. albicans CPK.2

S. cerevisiae jen1Δ p416-CaJen1-m presents a mediated transport system for lactic acid

Final Remarks

- *C. albicans* display activity for a monocarboxylates mediated transport system
- CaJen1p is induced by glycerol, lactic and pyruvic acids and is targeted to the plasma membrane
- In *S. cerevisiae CaJEN1* is expressed and the protein is targeted to the plasma membrane
- *CaJEN1* encodes for a monocarboxylate permease in yeast *C. albicans*

References

Casal, M., Paiva, S., Andrade, R. P., Gancedo, C. and Leão, C. (1999). The lactate-proton symport of *Saccharomyces cerevisiae* is encoded by *JEN1*. *J Bacteriol* 181, 2620-3.

Grobler, J., Bauer, F., Subden, R.E. and Van Vuuren, H.J. (1995) The *mae1* gene of *Schizosaccharomyces pombe* encodes a permease for malate and other C4 dicarboxylic acids. *Yeast*, 11, 1485-91.

Santos, M. A., Keith, G. and Tuite, M. F. (1993). Non-standard translational events in *Candida albicans* mediated by an unusual seryl-tRNA with a 5'-CAG-3' (leucine) anticodon. *Embo J* 12, 607-616.

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